ELECTRON MICROSCOPIC DETERMINATION OF SIZE AND SHAPE OF A NEW PLANT VIRUS (SANN HEMP MOSAIC)

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Plates A, B, XVIII

ABSTRACT. The size and shape of a new plant virus, *viz.*, sann hemp mosaic, has been determined with the electron microscope. The sample was obtained from a crystalline preparation of the material and was shadowed with gold for electron microscopy. The virus has been found to consist of spherical particles with a mean diameter of 37·4 ± 6.$m\mu$.

INTRODUCTION

The electron microscope has found one of its most important applications in the study of viruses. These bodies, responsible for many diseases of men, animals, plants and bacteria, range in size from about 300 $m\mu$ to 10 $m\mu$. Since the limit of resolution of microscopes using visible radiation is about 175-220 $m\mu$ and that of the ultra-violet microscope about 100 $m\mu$, only a few of the larger viruses could be directly photographed with these microscopes. Size determination for the smaller viruses was, therefore, based on such indirect measurements as filterability, sedimentation and diffussion rate, viscosity, X-ray study, etc. Most of these estimates were based on assumptions, the validity of which was far from certain.

Thus, the method based on filterability depended on the assumption that the filter membranes had uniform pore size and that the particles were all spherical. The method, therefore, could give only approximate sizes.

The sedimentation and diffusion rate measurements provide accurate results only if the preparations are homogeneous as regards particle size and weight and moreover, if certain assumptions are made, *viz.*, that Stoke's law holds for the sedimentation of the virus particles, that these particles are smooth, unhydrated spheres, and that the solution is structureless and has no anomalous physical properties. To what extent these assumptions are valid, is very uncertain and is itself a matter for further experimental investigation.

Size determination from viscosity measurements has been made by several investigators who have, however, given divergent values for the same specimen. With regard to these measurements we can do no better than quote Bawden (1949) who observes, "there is no reason to believe that any

of these measurements were made on homogeneous preparations; all preparations contained aggregated particles and the kindest interpretation that can be put on different estimates is to assume that preparations of different states of aggregation were being studied".

X-ray analysis, while it provides accurate size estimation, is unfortunately applicable only to those viruses that form crystals, or liquid crystals, for it depends on the refraction of X-rays by regularly arranged layers of particles.

Other methods of indirect determination of size, *viz.*, stream double refraction, fluorescence microscopy, radio sensitivity, etc., give such widely varying values for any particular virus that one is at a loss to decide about the correct size. Thus, in the case of tobacco mosaic virus, the most studied of all the viruses, the length measurements based on different methods range from 1400 $m\mu$ (from bi-refringence) to 270 $m\mu$ (light scattering) (Bawden, 1950).

The electron microscope, with its present limit of resolution of the order of 1 $m\mu$, provides a direct method of studying the shape and size of most of the viruses. Covering the entire range of sizes occupied by these bodies, the instrument has already proved, and will doubtless continue to prove, of the greatest value in such investigations.

The present paper gives an account of the electron microscopic determination of the size and shape of a new plant virus causing the mosaic disease of sann hemp plant. The virus was isolated and crystallised following the method due to Markham and Smith (1946) at the Indian Agricultural Research Institute, at Delhi (Raychaudhury, 1947). The present work was carried out with the help of the electron microscope installed at the Institute of Nuclear Physics, a detailed description of which has already been published (Dasgupta, and others 1948).

EXPERIMENTAL METHOD

For electron microscopy, the crystalline preparation of the sample was diluted in distilled water and a drop of the liquid was deposited on a thin collodion film supported by a 200-mesh wire screen. After allowing sufficient time for the suspended particles to settle down and adhere on to the collodion film, the excess water was drained off.

The sample thus prepared, when examined under the electron microscope gave very poor image contrast in the electron micrographs due to the low electron scattering power of the virus particles. To enhance the contrat of the micrographs, recourse was taken to metallic shadow-casting of the preparation (Wyckoff, 1949).

As usual, the specimen on collodion film was shadowed with an oblique beam of gold atoms in high vacuum. The angle of deposition of metal vapour was varied from $\tan^{-1} 1/10$ to $\tan^{-1} 1/12$. Such shadowed specimens were then micrographed. Three of the electron micrographs are reproduced

Fig

Fig 2.

Electron micrographs of gold-shadowed sann hemp mosaic virus.

ig. 1. { Shadowing angle : tan $^{-1}\frac{1}{2}$
Thickness of gold : 6.3 A.U.
Magnification : 38, 300 X

Fig. 2. { Shadowing angle : tan $^{-1}\frac{1}{10}$
Thickness of gold : 12.3 A.U.
Magnification : 45,000 X

Fig. 3

Electron micrograph of gold-shadowed sann hemp mosaic virus.
Shadowing angle : $\tan^{-1}\frac{1}{10}$;
Thickness of gold : 6.3 A.U.
Magnification : 40,800 X

in figures 1 and 2 of Plate XVIII A and figure 3 of Plate XVIII B. They show a greatly increased contrast due to enhanced scattering of electrons from the layer of metallic vapour deposited on the virus particles and the corresponding absence of the metal from the shadow regions. In addition to increasing contrast and thereby increasing the accuracy with which measurements can be made, shadowing further creates the impression that one is seeing the specimen in three dimension which greatly helps one to make an estimate of the shape of the particles.

Calibration of magnification.

In order to assess the size correctly from the micrographs, it was decided to calibrate the magnification of the instrument with the help of a collodion replica of a grating of known constant. This was done by keeping the projector current at the settings at which the virus micrographs had been taken and a grating replica was micrographed by mounting the replica sample at different distances from the objective pole piece. The corresponding objective lens focusing currents were recorded for the different mounting positions. From measurement of the line separations in the grating micrographs and knowing the grating constant, the total magnification at different objective currents were calculated and these magnifications were plotted against objective currents. From this curve of magnification *vs.*, objective current, the magnification of the virus micrographs at any specified objective current could be found out.

RESULTS

An inspection of the micrographs reveals that the predominating unit is essentially spherical in shape. The diameter of these units were carefully

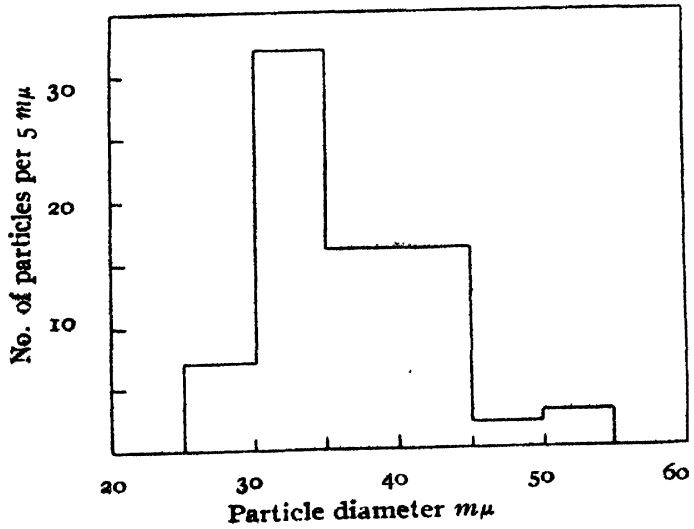

FIG. 4

Histogram illustrating the distribution of particle size in the micrographs of figures 1, 2 and 3.

measured and their actual sizes then deduced from the previously determined magnification factors. The spread of the measured particle diameters is illustrated by the histogram shown in figure 4. The mean diameter obtained is 37.4 $m\mu$ with a standard deviation of $\pm$ 6.1 $m\mu$.

The micrographs show the presence of a few bodies larger than the average size particles. These are, presumably, clusters of the elementary particles, which, however, due to insufficient resolution, could not be seen as distinctly separate bodies.

For the purpose of comparison with the other plant viruses studied with the electron microscope, the following table has been prepared showing the size and shape of all the plant viruses including the one studied in the present investigation :

TABLE I

Name of virus.	Shape	Size
1. Tobacco mosaic	Rod like	280 $m\mu$ long 15 $m\mu$ wide
2. Potato virus X	Rod like	500-600 $m\mu$ long 16 $m\mu$ wide
3. Southern bean mosaic	Spherical	26 $m\mu$ diam.
4. Tomato bushy stunt	Spherical	26 $m\mu$ diam.
5. Cucumber mosaic	Rod like	13 $m\mu$ wide.
6. Squash mosaic	Spherical	30 $m\mu$ diam.
7. Potato yellow dwarf	Rod like	200 $m\mu$ long 50 $m\mu$ wide
8. Tobacco necrosis	Spherical	20 $m\mu$ diam.
9. Turnip yellow mosaic	Spherical	22 $m\mu$ diam.
10. Sann hemp mosaic	Spherical	37 $m\mu$ diam.

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